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LETTER TO THE EDITOR

Response to letter to editor regarding Variability of symmetric dimethylarginine in apparently healthy dogs

Dear Editors,

We thank Drs Baral and Freeman for their comments and insights on this article. They highlight many of the issues that the veterinary profession will wrestle with as we develop a greater understanding of the role of biological variability and the interpretation of the same in clinical application.

Drs Baral and Freeman commented regarding the use of the term reference change value (RCV) rather than critical difference (C_D). We agree that consistency in terminology is desirable and that relative rather than absolute threshold values for determining that a patient has deviated from a previous state are more useful in a clinical context, but we would point out that the terms C_D and RCV are used synonymously throughout much of the biological variation literature. We accept the criticism that CD% of mean is a clumsy term that equates to RCV, and thus, we have used RCV values throughout the remainder of this response.

In a recent publication,[1] we made a mathematical error in the calculation of C_D and RCV, and hence, we have recalculated these values for the study cohort means for symmetric dimethylarginine (SDMA) and creatinine and found them to be 5.98 μg/dL (RCV of 47%) and 22.8 μmol/L (RCV 23.7%), respectively. It is of note that these recalculated C_D and RCV values indicate greater biological and analytical variability for these variables. Hence, for any given pair of SDMA and creatinine values, if the difference between the 2 measurements is more than 47% and 23.7% of the greater value, respectively, the probability that this difference is because of biological and laboratory variability is small and hence should be regarded as clinically significant. We regret this error and any confusion that may have arisen as a result.

Because our previously reported erroneous RCV for creatinine was so small (ie, 0.93%), the probability that any 2 consecutive creatinine measurements in our study population would have a difference this small was minute. This lead to our statement that none of our dogs had stable serum creatinine, because they all had at least 2 measurements that were above the originally calculated RCV. However, the new value calculated for RCV (23.7%) indicates that this statement was in error.

The authors additionally make a very valid comment regarding the analytical variability of the SDMA assay used in our study. Published guidelines for acceptable analytical variability in studies of biological variability and derivation of RCV values suggest that CV_A should be <1/2 of the coefficient of variation in the variability component of interest.[2] In the case of serial determinations within an individual patient, the important variability component is the within-individual variability, that is, CV_I. In our study population, the analytical variance of the SDMA assay was approximately 9.5%, a value that is more than 1/2 of the CV_I of our subjects for this analyte (14%). In our study population at least, the analytical variability was too great to fulfill these criteria for reporting of RCV values. However, we would argue that it was important to report at least an estimate of the RCV value for this assay, recognizing that a large proportion of this value is because of analytical variability. This assay has been explicitly recommended as a modality for the monitoring of progression of renal disease in companion animals, the very situation where an understanding of the RCV is important.

Analytical variability of 9.5% meets generally acceptable criteria for clinical use of ELISA-based assays,[3] and our value is consistent with a previously reported value for analytical precision of the SDMA immunoassay currently available commercially (reported in abstract form as ≤10%, American Association for Clinical Chemistry 2015, Abstract #B-048). These values are, however, notably higher than previously reported for liquid chromatography–mass spectrometry (LC-MS) assays of SDMA in veterinary species.[4] Bearing this in mind, if the RCV for SDMA in dogs were to be recalculated using the LC-MS analytical variance of 2.5% previously reported by one group[4] and our CV_I of 14%, the new RCV would approximately be 39% (as opposed to 47%). At the first glance, we consider an 8% difference between these 2 RCV values to have little likely impact on the interpretation of test results from this assay. We think that it is important to recognize that, at least in the apparently healthy dogs in our study group, a substantial proportion of changes in serum SDMA concentrations on serial sampling is because of analytical variability regardless of the assay method used, and this must be considered in the interpretation of results from this assay regardless of methodology.

The authors additionally comment on our sampling intervals, which varied in this study. We subsequently calculated the RCVs for SDMA and creatinine from our study using samples drawn only from the 2-, 7-, and 14-day intervals (values of 53%, 38%, and 53%, respectively) compared to the whole study RCV (47%). The RCVs for creatinine for the 2-, 7-, and 14-day intervals were 18%, 27%, and 23% respectively, compared to the whole study RCV of

23.7%. As suggested by the authors, there is a range of biological variabilities observed that vary with the interval. However, we do not necessarily agree that a fixed 7-day interval intrinsically provides a better estimation of the biological variability than any other interval. In our calculation above, recognizing that the precision of these estimates is limited by smaller sample sizes, the highest RCV value for creatinine is in the 7-day interval samples while the RCV for SDMA is the lowest with the same sampling interval. We suggest that a better approach for generating RCV values relevant to clinical application would be to determine the most likely interval that would be appropriate from the standpoint of clinical application, and use that interval, rather than using a fixed interval that "fits all tests." In one recent review article regarding study design for estimating biological variation in human medical practice, the authors state that "the sample time interval and the study duration should be related to retesting times used for the measurements of the specific analyte in clinical practice."[5] Much like creatinine, SDMA concentrations may be used for both monitoring of short-term response with acute kidney injury and for longer term monitoring of chronic renal insufficiency. Data regarding RCV in both of these scenarios are valuable.

Lastly, we agree with the authors that, in the interests of uniformity between studies, future studies should use the reciprocal formula when calculating the index of individuality.

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